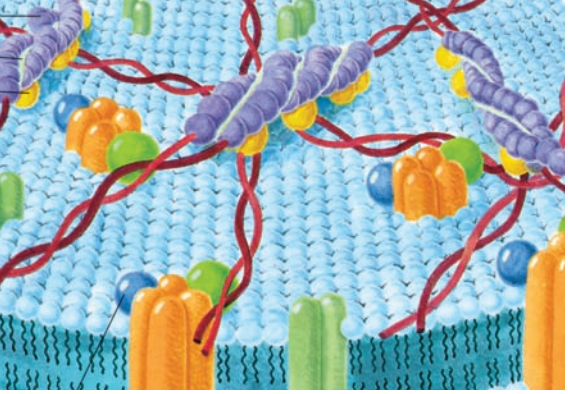

Lipids and Membranes

CHAPTER 12

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- B. Triacylglycerols
- C. Glycerophospholipids
- D. Sphingolipids
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Membranes function to organize biological processes by compartmentalizing them. Indeed, the cell, the basic unit of life, is essentially defined by its enveloping plasma membrane. Moreover, in eukaryotes, many subcellular organelles, such as nuclei, mitochondria, chloroplasts, the endoplasmic reticulum, and the Golgi apparatus (Fig. 1-5), are likewise membrane bounded.

Biological membranes are organized assemblies of lipids and proteins with small amounts of carbohydrate. Yet they are not impermeable barriers to the passage of materials. Rather, they regulate the composition of the intracellular medium by controlling the flow of nutrients, waste products, ions, etc., into and out of the cell. They do this through membrane-embedded "pumps" and "gates" that transport specific substances against an electrochemical gradient or permit their passage with such a gradient (Chapter 20).

Many fundamental biochemical processes occur on or in a membranous scaffolding. For example, electron transport and oxidative phosphorylation (Chapter 22), processes that oxidize nutrients with the concomitant generation of ATP, are mediated by an organized battery of enzymes that are components of the inner mitochondrial membrane. Likewise, photosynthesis, in which light energy powers the chemical combination of H_2O and CO_2 to form carbohydrates (Chapter 24), occurs in the inner membranes of chloroplasts. The processing of information, such as sensory stimuli or intercellular communications, is generally a membrane-based phenomenon. Thus nerve impulses are mediated by nerve cell membranes (Section 20-5) and the presence of certain substances such as hormones and nutrients is detected by specific membrane-bound receptors (Chapter 19).

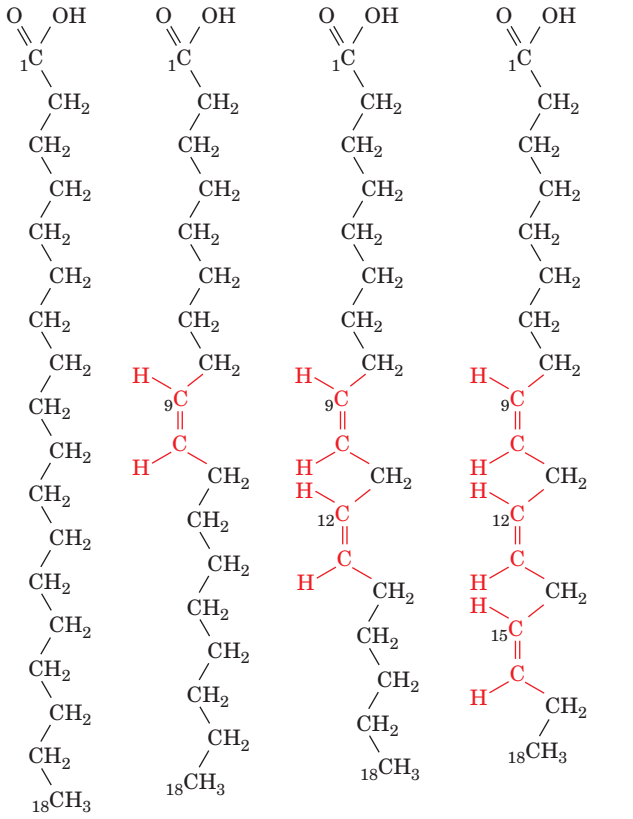
In this chapter, we examine the compositions, structures, and formation of biological membranes and related substances. Specific membrane-based biochemical processes, such as those mentioned above, are dealt with in later chapters.

1 LIPID CLASSIFICATION

Lipids *(Greek: lipos, fat) are substances of biological origin that are soluble in organic solvents such as chloroform and methanol but are only sparingly soluble, if at all, in water.* Hence, they are easily separated from other biological materials by extraction into organic solvents and may be further fractionated by such techniques as adsorption chromatography, thin layer chromatography, and reverse-phase chromatography (Section 6-3D). Fats, oils, certain vitamins and hormones, and most nonprotein membrane components are lipids. In this section, we discuss the structures and physical properties of the major classes of lipids.

A. *Fatty Acids*

Fatty acids *are carboxylic acids with long-chain hydrocarbon side groups* (Fig. 12-1). They are rarely free in nature but, rather, occur in esterified form as the major components of the various lipids described in this chapter. The more common biological fatty acids are listed in Table 12-1. In higher plants and animals, the predominant fatty acid residues are those of the C_{16} and C_{18} species **palmitic, oleic, linoleic,** and **stearic acids.** Fatty acids with <14 or >20 carbon atoms are uncommon. *Most fatty acids have an even number of carbon*



Stearic acid **Oleic acid** **Linoleic acid** **α-Linolenic acid**

Figure 12-1 **Structural formulas of some C_{18} fatty acids.** The double bonds all have the cis configuration.

atoms because they are usually biosynthesized by the concatenation of C_2 units (Section 25-4C). Over half of the fatty acid residues of plant and animal lipids are unsaturated (contain double bonds) and are often polyunsaturated (contain two or more double bonds). Bacterial fatty acids are rarely polyunsaturated but are commonly branched, hydroxylated, or contain cyclopropane rings. Unusual fatty acids also occur as components of the oils and **waxes** (esters of fatty acids and long-chain alcohols) produced by certain plants.

a. The Physical Properties of Fatty Acids Vary with Their Degree of Unsaturation

Table 12-1 indicates that the first double bond of an unsaturated fatty acid commonly occurs between its C9 and C10 atoms counting from the carboxyl C atom (a Δ^9- or 9-double bond). In polyunsaturated fatty acids, the double bonds tend to occur at every third carbon atom toward the methyl terminus of the molecule (such as —CH═CH—CH_2—CH═CH—). Double bonds in polyunsaturated fatty acids are almost never conjugated (as in —CH═CH—CH═CH—). Triple bonds rarely occur in fatty acids or any other compound of biological origin. Two important classes of polyunsaturated fatty acids are denoted $n-3$ (or $\omega-3$) and $n-6$ (or $\omega-6$) fatty acids. This nomenclature identifies the last double-bonded carbon atom as counted from the methyl terminal (ω) end of the chain.

Saturated fatty acids are highly flexible molecules that can assume a wide range of conformations because there is relatively free rotation about each of their C—C bonds. Nevertheless, their fully extended conformation is that of minimum energy because this conformation has the least amount of

Table 12-1 **The Common Biological Fatty Acids**

Symbol[a]	Common Name	Systematic Name	Structure	mp (°C)
Saturated fatty acids				
12:0	Lauric acid	Dodecanoic acid	$CH_3(CH_2)_{10}COOH$	44.2
14:0	Myristic acid	Tetradecanoic acid	$CH_3(CH_2)_{12}COOH$	52
16:0	Palmitic acid	Hexadecanoic acid	$CH_3(CH_2)_{14}COOH$	63.1
18:0	Stearic acid	Octadecanoic acid	$CH_3(CH_2)_{16}COOH$	69.6
20:0	Arachidic acid	Eicosanoic acid	$CH_3(CH_2)_{18}COOH$	75.4
22:0	Behenic acid	Docosanoic acid	$CH_3(CH_2)_{20}COOH$	81
24:0	Lignoceric acid	Tetracosanoic acid	$CH_3(CH_2)_{22}COOH$	84.2
Unsaturated fatty acids (all double bonds are cis)				
16:1*n*–7	Palmitoleic acid	9-Hexadecenoic acid	$CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$	−0.5
18:1*n*–9	Oleic acid	9-Octadecenoic acid	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$	13.4
18:2*n*–6	Linoleic acid	9,12-Octadecadienoic acid	$CH_3(CH_2)_4(CH{=}CHCH_2)_2(CH_2)_6COOH$	−9
18:3*n*–3	α-Linolenic acid	9,12,15-Octadecatrienoic acid	$CH_3CH_2(CH{=}CHCH_2)_3(CH_2)_6COOH$	−17
18:3*n*–6	γ-Linolenic acid	6,9,12-Octadecatrienoic acid	$CH_3(CH_2)_4(CH{=}CHCH_2)_3(CH_2)_3COOH$	
20:4*n*–4	Arachidonic acid	5,8,11,14-Eicosatetraenoic acid	$CH_3(CH_2)_4(CH{=}CHCH_2)_4(CH_2)_2COOH$	−49.5
20:5*n*–3	EPA	5,8,11,14,17-Eicosapentaenoic acid	$CH_3CH_2(CH{=}CHCH_2)_5(CH_2)_2COOH$	−54
22:6*n*–3	DHA	4,7,10,13,16,19-Docosahexenoic acid	$CH_3CH_2(CH{=}CHCH)_6CH_2COOH$	
24:1*n*–9	Nervonic acid	15-Tetracosenoic acid	$CH_3(CH_2)_7CH{=}CH(CH_2)_{13}COOH$	39

[a]Number of carbon atoms: number of double bonds. For unsaturated fatty acids, n is the number of carbon atoms, $n-x$ is the double-bonded carbon atom, and x is the number of that carbon atom counting from the methyl terminal (ω) end of the chain.

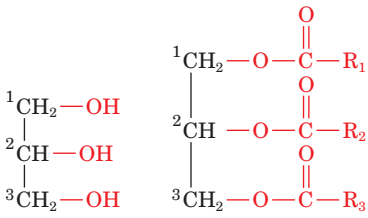
Source: Dawson, R.M.C., Elliott, D.C., Elliott, W.H., and Jones, K.M., *Data for Biochemical Research* (3rd ed.), Chapter 8, Clarendon Press (1986).

steric interference between neighboring methylene groups. The melting points (mp) of saturated fatty acids, like those of most substances, increase with molecular mass (Table 12-1).

Fatty acid double bonds almost always have the cis configuration (Fig. 12-1). This puts a rigid 30° bend in the hydrocarbon chain of unsaturated fatty acids that interferes with their efficient packing to fill space. The consequent reduced van der Waals interactions cause fatty acid melting points to decrease with their degree of unsaturation (Table 12-1). Lipid fluidity likewise increases with the degree of unsaturation of their component fatty acid residues. This phenomenon, as we shall see in Section 12-2Cb, has important consequences for membrane properties.

B. *Triacylglycerols*

The fats and oils that occur in plants and animals consist largely of mixtures of **triacylglycerols** (also referred to as **triglycerides** or **neutral fats**). *These nonpolar, water-insoluble substances are fatty acid triesters of* **glycerol:**



Glycerol **Triacylglycerol**

Triacylglycerols function as energy reservoirs in animals and are therefore their most abundant class of lipids even though they are not components of biological membranes.

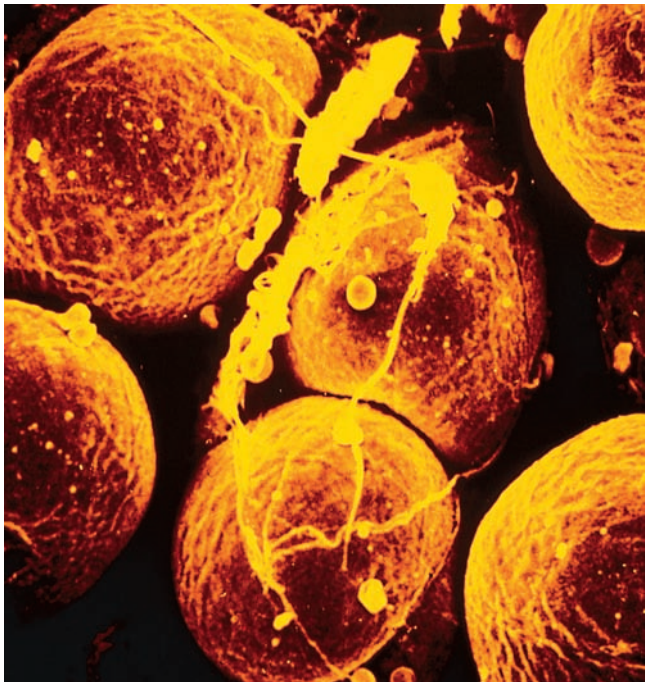
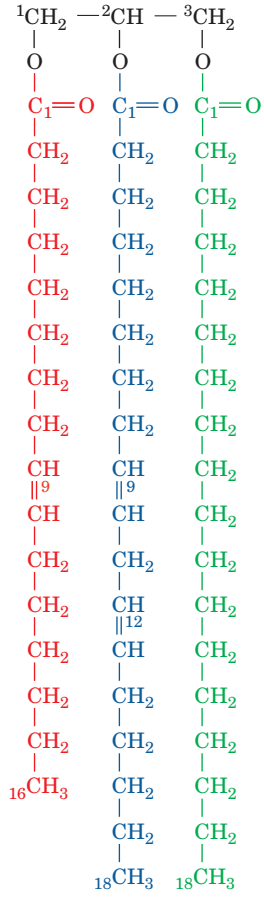


Figure 12-2 **Scanning electron micrograph of adipocytes.** Each contains a fat globule that occupies nearly the entire cell. [Fred E. Hossler/Visuals Unlimited.]

Triacylglycerols differ according to the identity and placement of their three fatty acid residues. The so-called **simple triacylglycerols** contain one type of fatty acid residue and are named accordingly. For example, **tristearoylglycerol** or **tristearin** contains three stearic acid residues, whereas **trioleoylglycerol** or **triolein** has three oleic acid residues. The more common **mixed triacylglycerols** contain two or three different types of fatty acid residues and are named according to their placement on the glycerol moiety.



1-Palmitoleoyl-2-linoleoyl-3-stearoylglycerol

Fats and oils (which differ only in that fats are solid and oils are liquid at room temperature) are complex mixtures of simple and mixed triacylglycerols whose fatty acid compositions vary with the organism that produced them. Plant oils are usually richer in unsaturated fatty acid residues than are animal fats, as the lower melting points of oils imply.

a. Triacylglycerols Are Efficient Energy Reserves

Fats are a highly efficient form in which to store metabolic energy. This is because fats are less oxidized than are carbohydrates or proteins and hence yield significantly more energy on oxidation. Furthermore, fats, being nonpolar substances, are stored in anhydrous form, whereas glycogen, for example, binds about twice its weight of water under physiological conditions. Fats therefore provide about six times the metabolic energy of an equal weight of hydrated glycogen.

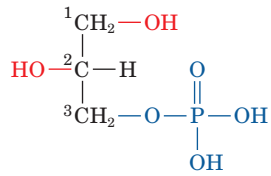
In animals, **adipocytes** (fat cells; Fig. 12-2) are specialized for the synthesis and storage of triacylglycerols. Whereas

other types of cells have only a few small droplets of fat dispersed in their cytosol, adipocytes may be almost entirely filled with fat globules. **Adipose tissue** is most abundant in a subcutaneous layer and in the abdominal cavity. The fat content of normal humans (21% for men, 26% for women) enables them to survive starvation for 2 to 3 months. In contrast, the body's glycogen supply, which functions as a short-term energy store, can provide for the body's metabolic needs for less than a day. The subcutaneous fat layer also provides thermal insulation, which is particularly important for warm-blooded aquatic animals, such as whales, seals, geese, and penguins, which are routinely exposed to low temperatures.

C. *Glycerophospholipids*

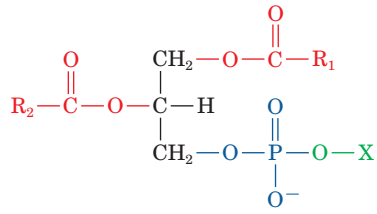
Glycerophospholipids *(or* ***phosphoglycerides****) are the major lipid components of biological membranes.* They consist of ***sn*-glycerol-3-phosphate** (Fig. 12-3*a*) esterified at its C1 and C2 positions to fatty acids and at its phosphoryl group to a group, X, to form the class of substances diagrammed in Fig. 12-3*b*. *Glycerophospholipids are therefore amphiphilic molecules with nonpolar aliphatic "tails" and polar phosphoryl-X "heads."* The simplest glycerophospholipids, in which X = H, are **phosphatidic acids;** they are present only in small amounts in biological membranes. *In the glycerophospholipids that commonly occur in biological membranes, the head groups are derived from polar alcohols* (Table 12-2). Saturated C_{16} and C_{18} fatty acids usually

(a)



***sn*-Glycerol-3-phosphate**

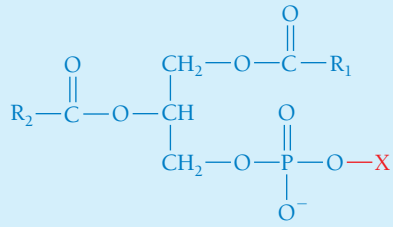
(b)

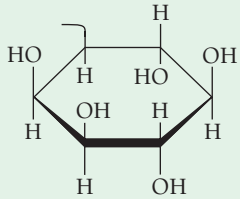
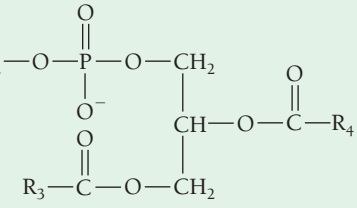


Glycerophospholipid

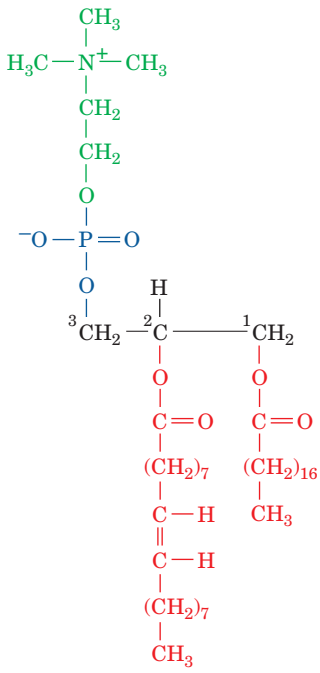
Figure 12-3 **Molecular formula of glycerophospholipids.** (*a*) The compound shown in Fischer projection (Section 4-2B) can be equivalently referred to as L-glycerol-3-phosphate or D-glycerol-1-phosphate. However, using **stereospecific numbering** (*sn*), which assigns the 1-position to the group occupying the *pro-S* position of a prochiral center (see Section 4-2Ca for a discussion of prochirality), the compound is unambiguously named *sn*-glycerol-3-phosphate. (*b*) The general formula of the glycerophospholipids. R_1 and R_2 are long-chain hydrocarbon tails of fatty acids and X is derived from a polar alcohol (Table 12-2).

Table 12-2 **The Common Classes of Glycerophospholipids**



Name of X—OH	Formula of —X	Name of Phospholipid
Water	—H	Phosphatidic acid
Ethanolamine	$-CH_2CH_2NH_3^+$	Phosphatidylethanolamine
Choline	$-CH_2CH_2N(CH_3)_3^+$	Phosphatidylcholine (lecithin)
Serine	$-CH_2CH(NH_3^+)COO^-$	Phosphatidylserine
myo-Inositol		Phosphatidylinositol
Glycerol	$-CH_2CH(OH)CH_2OH$	Phosphatidylglycerol
Phosphatidylglycerol		Diphosphatidylglycerol (cardiolipin)

(*a*)



1-Stearoyl-2-oleoyl-3-phosphatidylcholine

(*b*)

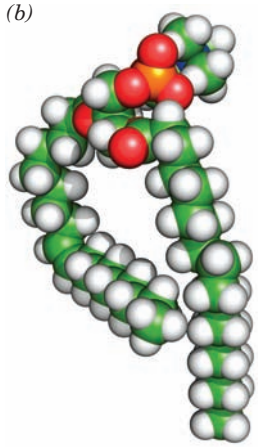
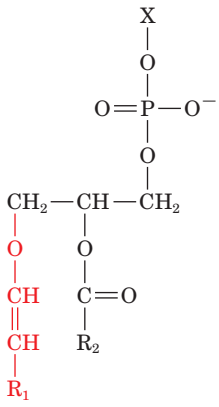


Figure 12-4 **The glycerophospholipid 1-stearoyl-2-oleoyl-3-phosphatidylcholine.** (*a*) Molecular formula in Fischer projection. (*b*) Energy-minimized space-filling model with C green, H white, N blue, O red, and P orange. Note how the unsaturated oleyl chain (*left*) is bent compared to the saturated stearoyl chain. [Based on coordinates provided by Richard Venable and Richard Pastor, NIH, Bethesda, Maryland.]

occur at the C1 position of glycerophospholipids, and the C2 position is often occupied by an unsaturated C_{16} to C_{20} fatty acid. Glycerophospholipids are, of course, also named according to the identities of these fatty acid residues (Fig. 12-4). Some glycerophospholipids have common names. For example, phosphatidylcholines are known as **lecithins;** diphosphatidylglycerols, the "double" glycerol phospholipids, are known as **cardiolipins** (because they were first isolated from heart muscle).

Plasmalogens

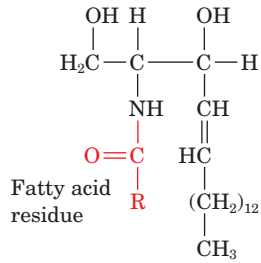


A plasmalogen

are glycerophospholipids in which the C1 substituent to the glycerol moiety is bonded to it via an α,β-unsaturated ether linkage in the cis configuration rather than through an ester linkage. **Ethanolamine, choline,** and serine form the most common plasmalogen head groups.

D. *Sphingolipids*

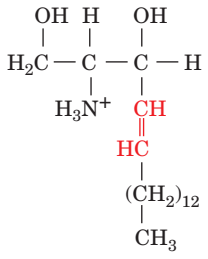
Sphingolipids, which are also major membrane components, are derivatives of the C_{18} amino alcohols **sphingosine, dihydrosphingosine** (Fig. 12-5), and their C_{16}, C_{17}, C_{19}, and C_{20} homologs. Their *N*-acyl fatty acid derivatives, **ceramides,**



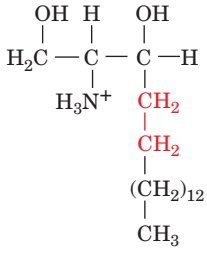
A ceramide

occur only in small amounts in plant and animal tissues but form the parent compounds of more abundant sphingolipids:

1. Sphingomyelins, the most common sphingolipids, are ceramides bearing either a phosphocholine (Fig. 12-6) or a phosphoethanolamine moiety, so that they can also be classified as **sphingophospholipids.** *Although sphingomyelins*



Sphingosine

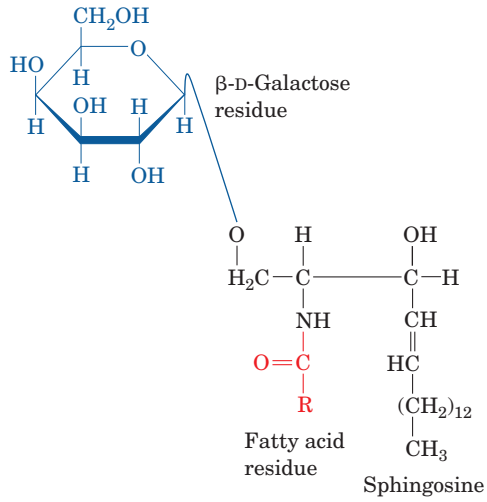


Dihydrosphingosine

Figure 12-5 **Molecular formulas of sphingosine and dihydrosphingosine.** The chiral centers at C2 and C3 of sphingosine and dihydrosphingosine have the configurations shown in Fischer projection. The double bond in sphingosine has the trans configuration.

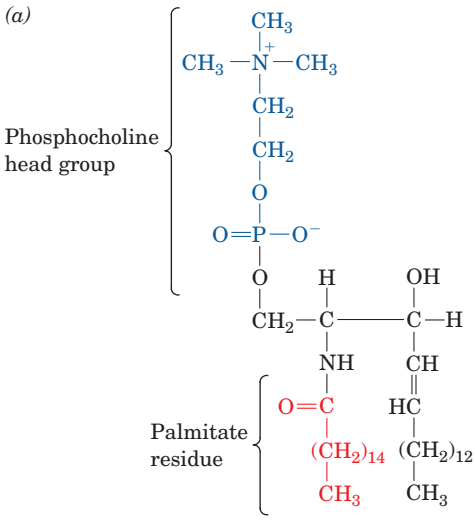
differ chemically from phosphatidylcholine and phosphatidylethanolamine, their conformations and charge distributions are quite similar. The membranous myelin sheath that surrounds and electrically insulates many nerve cell axons (Section 20-5Bc) is particularly rich in sphingomyelin.

2. Cerebrosides, the simplest **sphingoglycolipids** (alternatively **glycosphingolipids**), are ceramides with head groups that consist of a single sugar residue. **Galactocerebrosides,** which are most prevalent in the neuronal cell membranes of the brain, have a β-D-galactose head group.



A galactocerebroside

Glucocerebrosides, which instead have a β-D-glucose residue, occur in the membranes of other tissues. *Cerebrosides, in contrast to phospholipids, lack phosphate groups and hence are most frequently nonionic compounds.* The galactose residues of some galactocerebrosides, however, are sulfated at their C3 positions to form ionic compounds known as **sulfatides.** More complex sphingoglycolipids have unbranched oligosaccharide head groups of up to four sugar residues.



A sphingomyelin

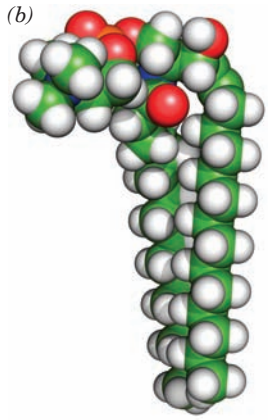


Figure 12-6 **A sphingomyelin.** (*a*) Molecular formula in Fischer projection. (*b*) Energy-minimized space-filling model with C green, H white, N blue, O red, and P orange. Note its conformational resemblance to glycerophospholipids (Fig. 12-4). [Based on coordinates provided by Richard Venable and Richard Pastor, NIH, Bethesda, Maryland.]

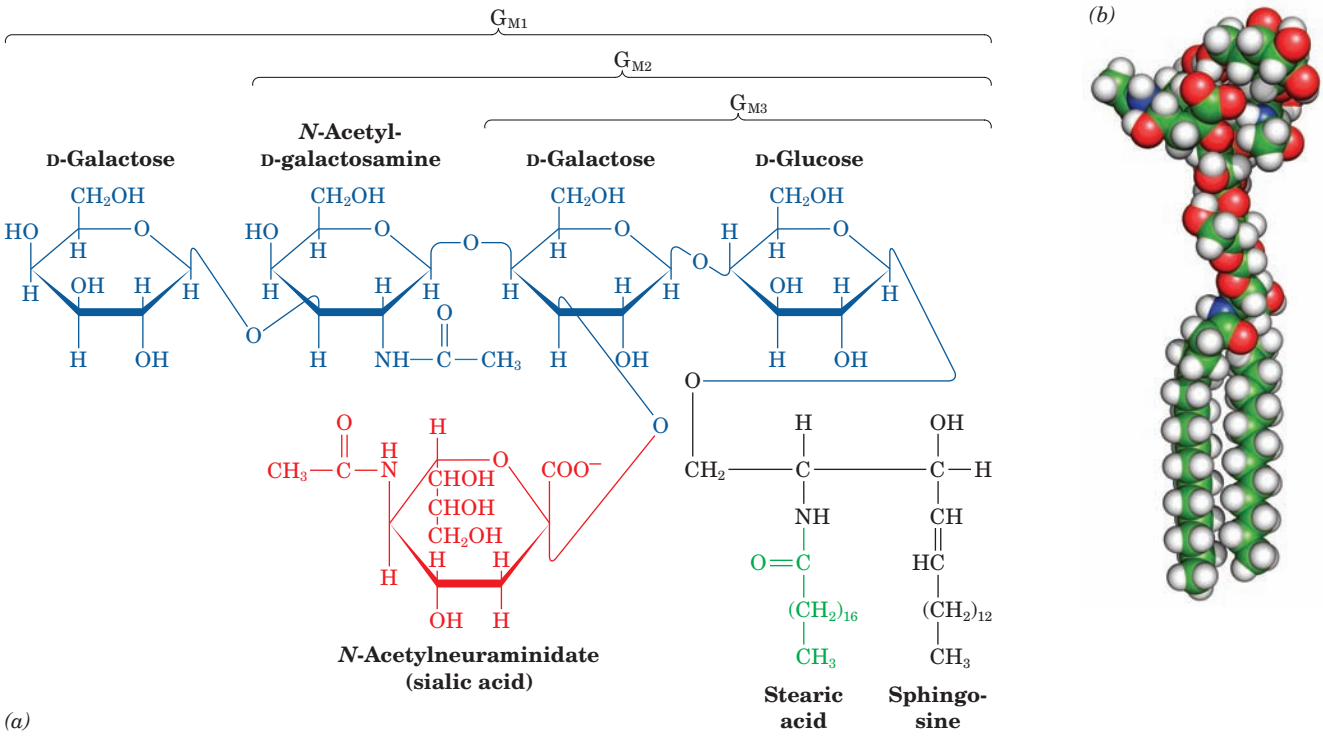
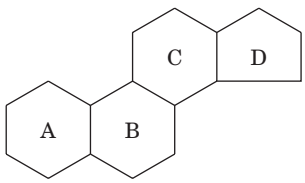


Figure 12-7 **Ganglioside G_{M1}.** (*a*) Structural formula with its sphingosine residue in Fischer projection. (*b*) Energy-minimized space-filling model with C green, H white, N blue, O red, and P orange. Gangliosides G_{M2} and G_{M3} differ from G_{M1} only by the sequential absences of the terminal D-galactose and *N*-acetyl-D-galactosamine residues. Other gangliosides have different oligosaccharide head groups. [Based on coordinates provided by Richard Venable and Richard Pastor, NIH, Bethesda, Maryland.]

3. Gangliosides form the most complex group of sphingoglycolipids. They are ceramide oligosaccharides that include among their sugar groups at least one sialic acid residue (*N*-acetylneuraminic acid and its derivatives; Section 11-1Cc). The structures of gangliosides G_{M1}, G_{M2}, and G_{M3}, three of the hundreds that are known, are shown in Fig. 12-7. Gangliosides are primarily components of cell-surface membranes and constitute a significant fraction (6%) of brain lipids. Other tissues also contain gangliosides but in lesser amounts.

Gangliosides have considerable physiological and medical significance. Their complex carbohydrate head groups, which extend beyond the surfaces of cell membranes, act as specific receptors for certain pituitary glycoprotein hormones that regulate a number of important physiological functions (Section 19-1). Gangliosides are also receptors for bacterial protein toxins such as cholera toxin (Section 19-2Cd). There is considerable evidence that gangliosides are specific determinants of cell–cell recognition, so they probably have an important role in the growth and differentiation of tissues as well as in carcinogenesis (cancer generation). Disorders of ganglioside breakdown are responsible for several hereditary **sphingolipid storage diseases,** such as **Tay-Sachs disease,** which are characterized by an invariably fatal neurological deterioration (Section 25-8Ce).



Cyclopentanoperhydrophenanthrene

Figure 12-8 **Cyclopentanoperhydrophenanthrene, the parent compound of steroids.** It consists of four fused saturated rings. The standard ring labeling system is indicated.

E. *Cholesterol*

Steroids, *which are mostly of eukaryotic origin, are derivatives of* ***cyclopentanoperhydrophenanthrene*** (Fig. 12-8). The much maligned **cholesterol** (Fig. 12-9), the most abundant steroid in animals, is further classified as a **sterol** because of its C3-OH group and its branched aliphatic side chain of 8 to 10 carbon atoms at C17.

Cholesterol is a major component of animal plasma membranes, where it is typically present at 30 to 40 mol %, and occurs in lesser amounts in the membranes of their subcellular organelles. Its polar OH group gives it a weak amphiphilic character, whereas its fused ring system provides it with greater rigidity than other membrane lipids. Cholesterol is therefore an important determinant of membrane

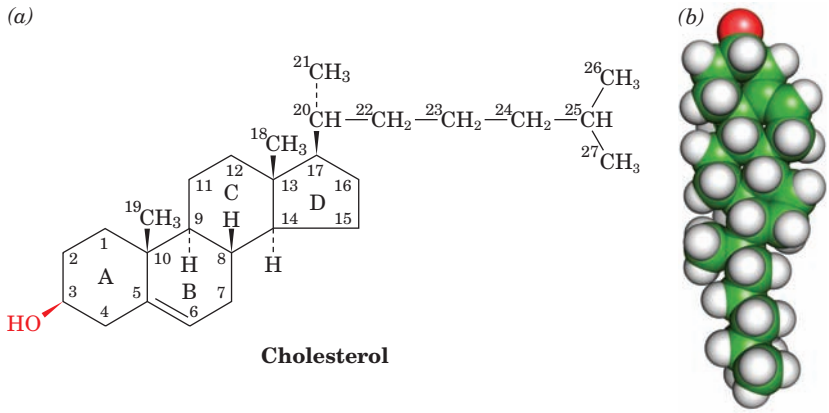
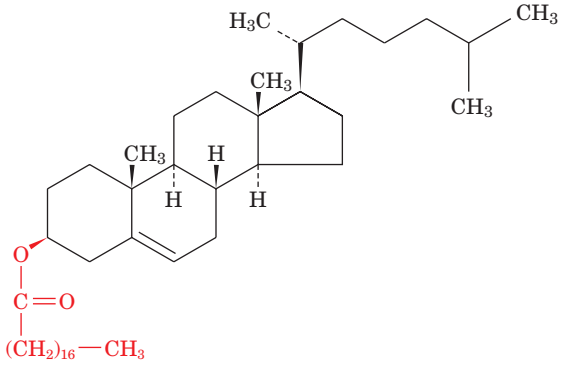


Figure 12-9 **Cholesterol.** (*a*) Structural formula with the standard numbering system. (*b*) Energy-minimized space-filling model with C green, H white, O red, and P orange. Cholesterol's rigid ring system makes it far less conformationally flexible than are membrane lipids: Its cyclohexane rings can adopt either the boat or the chair conformations (Fig. 11-6) but the chair conformation is highly preferred. [Based on coordinates provided by Richard Venable and Richard Pastor, NIH, Bethesda, Maryland.]

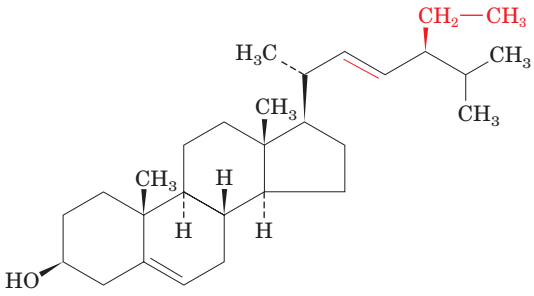
properties. It is also abundant in blood plasma lipoproteins (Section 12-5), where ~70% of it is esterified to long-chain fatty acids to form **cholesteryl esters.**



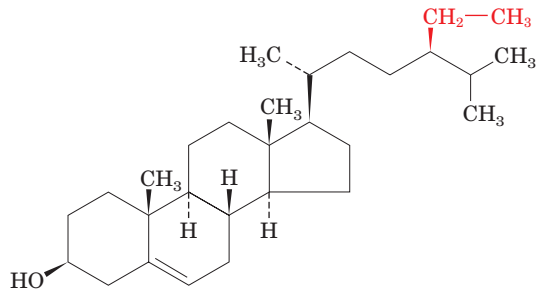
Cholesteryl stearate

Cholesterol is the metabolic precursor of **steroid hormones,** substances that regulate a great variety of physiological functions including sexual development and carbohydrate metabolism (Section 19-1G). The much-debated role of cholesterol in heart disease is examined in Section 12-5C. Cholesterol metabolism and the biosynthesis of steroid hormones are discussed in Section 25-6.

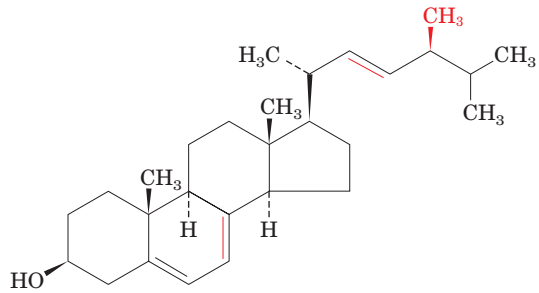
Plants contain little cholesterol. Rather, the most common sterol components of their membranes are **stigmasterol** and **β-sitosterol**



Stigmasterol



β-Sitosterol



Ergosterol

which differ from cholesterol only in their aliphatic side chains. Yeast and fungi have yet other membrane sterols such as **ergosterol,** which has a C7 to C8 double bond. Prokaryotes, with the exception of mycoplasmas (Section 1-1B), contain little, if any, sterol.

2 PROPERTIES OF LIPID AGGREGATES

The first recorded experiments on the physical properties of lipids were made in 1774 by the American statesman and scientist Benjamin Franklin. In investigating the well-known (at least among sailors) action of oil in calming waves, Franklin wrote:

> *At length being at Clapham [in London] where there is, on the common, a large pond, which I observed to be one day very*

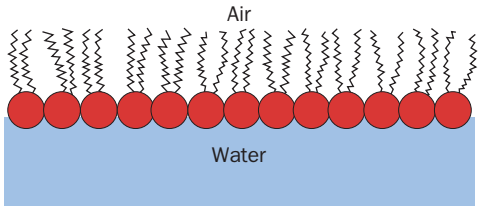


Figure 12-10 **An oil monolayer at the air–water interface.** The hydrophobic tails of the lipids avoid association with water by extending into the air.

> *rough with the wind, I fetched out a cruet of oil [probably olive oil] and dropt a little of it in the water. I saw it spread itself with surprising swiftness upon the surface. . . . I then went to the windward side, where [the waves] began to form; and there the oil, though not more than a teaspoonful, produced an instant calm over a space several yards square, which spread amazingly, and extended itself gradually till it reached the lee side, making all that quarter of the pond, perhaps half an acre, as smooth as a looking glass.*

This is sufficient information to permit the calculation of the oil layer's thickness (although there is no indication that Franklin made this calculation, we can; see Problem 4). We now know that oil forms a monomolecular layer on the surface of water in which the polar heads of the amphiphilic oil molecules are immersed in the water and their hydrophobic tails extend into the air (Fig. 12-10).

The calming effect of oil on rough water is a consequence of a large reduction in the water's surface tension. An oily surface film has the weak intermolecular cohesion characteristic of hydrocarbons rather than the strong intermolecular attractions of water responsible for its normally large surface tension. Oil, nevertheless, calms only smaller waves; it does not, as Franklin later observed, affect the larger swells.

In this section, we discuss how lipids aggregate to form micelles and bilayers. We shall also be concerned with the physical properties of lipids in bilayers because these aggregates form the structural basis for biological membranes.

A. *Micelles and Bilayers*

In aqueous solutions, amphiphilic molecules, such as soaps and detergents, form micelles (globular aggregates whose hydrocarbon groups are out of contact with water; Section 2-1Ba). This molecular arrangement eliminates unfavorable contacts between water and the hydrophobic tails of the amphiphiles and yet permits the solvation of the polar head groups. Micelle formation is a cooperative process: An assembly of just a few amphiphiles cannot shield its tails from contact with water. Consequently, dilute aqueous solutions of amphiphiles do not form micelles until their concentration surpasses a certain **critical micelle concentration (cmc).** Above the cmc, almost all the added amphiphile aggregates to form micelles. The value of the cmc depends on the identity of the amphiphile and the solution conditions. For amphiphiles with relatively small single tails, such as dodecyl sulfate ion, $CH_3(CH_2)_{11}OSO_3^-$, the cmc is $\sim$1 m*M*. Those of biological lipids, most of which have two large hydrophobic tails, are generally $<10^{-6}$ *M*.

a. Single-Tailed Lipids Tend to Form Micelles

The approximate size and shape of a micelle can be predicted from geometrical considerations. Single-tailed amphiphiles, such as soap anions, form spheroidal or ellipsoidal micelles because of their conical shapes (their hydrated head groups are wider than their tails; Fig. 12-11*a,b*). The number of molecules in such micelles depends on the amphiphile, but for many substances, it is on the order of several hundred. For a given amphiphile, these numbers span a narrow range: Less would expose the hydrophobic core of the micelle to water, whereas more would give the micelle an energetically unfavorable hollow center (Fig. 12-11*c*). Of course, a large micelle could flatten out to eliminate this hollow center, but the resulting decrease of curvature at the flattened surfaces would also generate empty spaces (Fig. 12-11*d*).

b. Glycerophospholipids and Sphingolipids Tend to Form Bilayers

The two hydrocarbon tails of glycerophospholipids and sphingolipids give these amphiphiles a more or less cylindrical

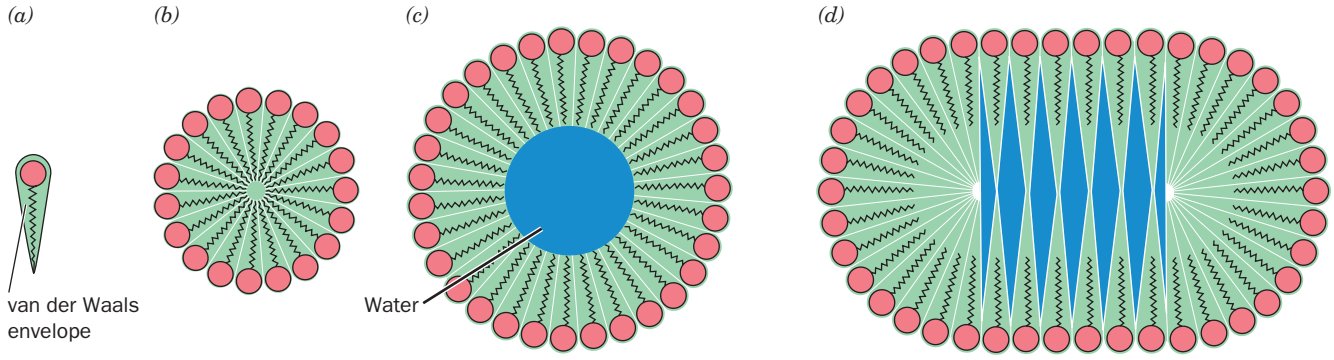


Figure 12-11 **Aggregates of single-tailed lipids.** The conical van der Waals envelope of single-tailed lipids (*a*) permits them to pack efficiently in forming a spheroidal micelle (*b*). The diameters of these micelles and hence their lipid population largely depend on the length of the tails. Spheroidal micelles composed of many more lipid molecules than the optimal number would have an unfavorable water-filled center (*blue*) (*c*). Such micelles could flatten out to collapse the hollow center, but as such ellipsoidal micelles become elongated they also develop water-filled spaces (*d*).

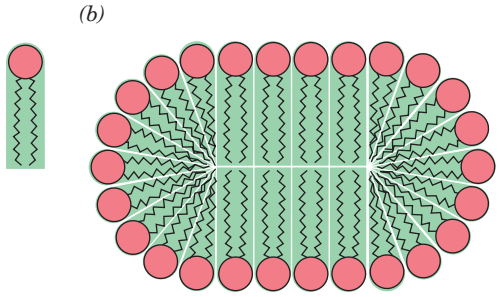
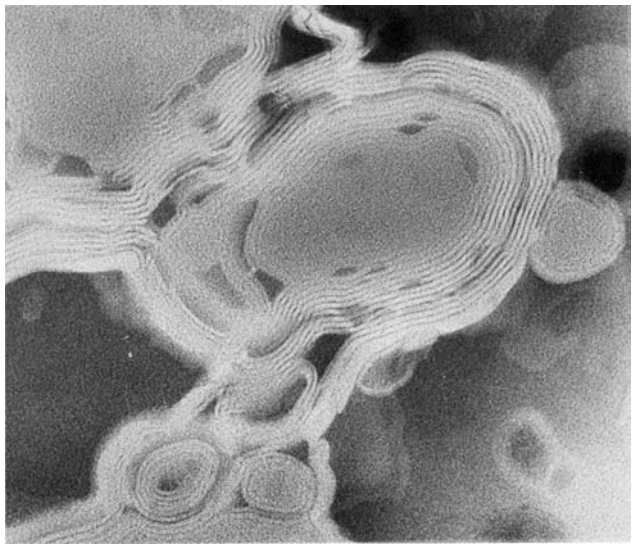
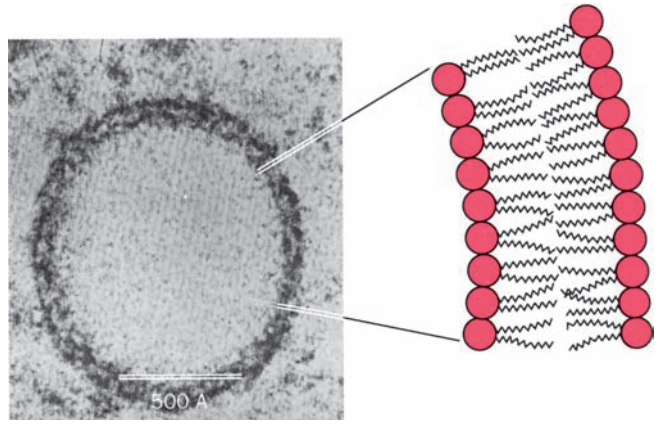


Figure 12-12 **Bilayer formation by phospholipids.** The cylindrical van der Waals envelope of phospholipids (*a*) causes them to form extended disklike micelles (*b*) that are better described as lipid bilayers.

shape (Fig. 12-12*a*). The steric requirements of packing such molecules together yields large disklike micelles (Fig. 12-12*b*) that are really extended bimolecular leaflets. The existence of such **lipid bilayers** was first proposed in 1925 by Evert Gorter and François Grendel, on the basis of their observation that lipids extracted from erythrocytes covered twice the area when spread as a monolayer at the air–water interface (Fig. 12-10) than in the erythrocyte plasma membrane (the erythrocyte's only membrane). Lipid bilayers typically have thicknesses of ~60 Å, as measured by electron microscopy and X-ray diffraction techniques. Since their two head group layers are each expected to be ~15 Å thick, their ~15-Å-long hydrocarbon tails must be nearly fully extended. We shall see below that *lipid bilayers form the structural basis of biological membranes.*



(a)



(b)

Figure 12-13 **Lipid bilayers.** (*a*) An electron micrograph of a multilamellar phospholipid vesicle in which each layer is a lipid bilayer. [Courtesy of Alec D. Bangham, Institute of Animal Physiology, Cambridge, U.K.] (*b*) An electron micrograph of a liposome. Its wall, as the accompanying diagram indicates, consists of a bilayer. [Courtesy of Walter Stoeckenius, University of California at San Francisco.]

B. *Liposomes*

A suspension of phospholipids in water forms multilamellar vesicles that have an onionlike arrangement of lipid bilayers (Fig. 12-13*a*). On **sonication** (agitation by ultrasonic vibrations), these structures rearrange to form **liposomes**—closed, self-sealing, solvent-filled vesicles that are bounded by only a single bilayer (Fig. 12-13*b*). They usually have diameters of several hundred Ångstroms and, in a given preparation, are rather uniform in size. Liposomes with diameters of ~1000 Å can be made by injecting an ethanolic solution of phospholipid into water or by dissolving phospholipid in a detergent solution and then dialyzing out the detergent. Once formed, liposomes are quite stable and, in fact, may be separated from the solution in which they reside by dialysis, gel filtration chromatography, or centrifugation. Liposomes with differing internal and external environments can therefore be readily prepared. *Biological membranes consist of lipid bilayers with which proteins are associated (Section 12-3A).* Liposomes composed of synthetic lipids and/or lipids extracted from biological sources (e.g., lecithin from egg yolks) have therefore been extensively studied as models for biological membranes.

a. Lipid Bilayers Are Impermeable to Most Polar Substances

Since biological membranes form cell and organelle boundaries, it is important to determine their ability to partition two aqueous compartments. The permeability of a lipid bilayer to a given substance may be determined by forming liposomes in a solution containing the substance of interest, changing the external aqueous solution, and then measuring the rate at which the substance appears in the new external solution. It has been found in this way that *lipid bilayers are extraordinarily impermeable to ionic and polar substances and that the permeabilities of such substances increase with their solubilities in nonpolar solvents.* This suggests that to penetrate a lipid bilayer, a solute molecule must shed its hydration shell and become solvated by

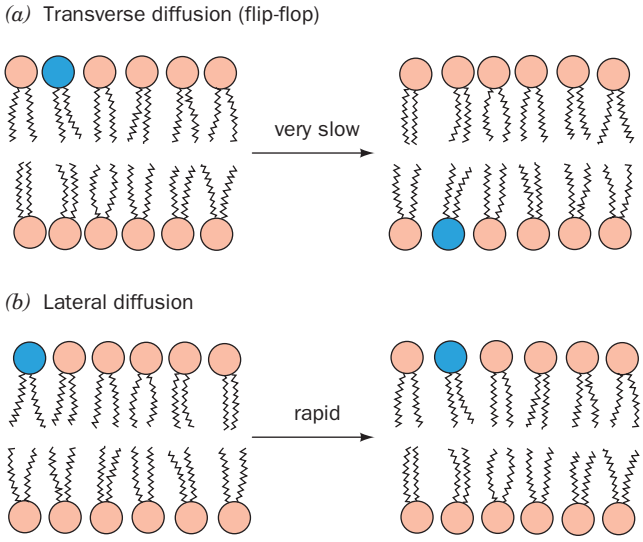


Figure 12-14 **Phospholipid diffusion in a lipid bilayer.** (*a*) Transverse diffusion (flip-flop) is defined as the transfer of a phospholipid molecule from one bilayer leaflet to the other. (*b*) Lateral diffusion is defined as the pairwise exchange of neighboring phospholipid molecules in the same bilayer leaflet.

the bilayer's hydrocarbon core. Such a process is highly unfavorable for polar molecules, so that even the ~30-Å thickness of a lipid bilayer's hydrocarbon core forms an effective barrier for polar substances. However, measurements using tritiated water indicate that lipid bilayers are appreciably permeable to water. Despite the polarity of water, its small molecular size makes it significantly soluble in the hydrocarbon core of lipid bilayers and therefore able to permeate them.

The stability of liposomes and their impermeability to many substances make them promising vehicles for the delivery of therapeutic agents, such as drugs, enzymes, and genes (for gene therapy), to particular tissues. Liposomes are absorbed by many cells through fusion with their plasma membranes. If methods can be developed for targeting liposomes to specific cell populations, then the desired substances could be directed toward particular tissues through liposome microencapsulation. Indeed, a number of liposome-delivered anticancer agents and antibiotics are already in use.

C. *Bilayer Dynamics*

a. Lipid Bilayers Are Two-Dimensional Fluids

The transfer of a lipid molecule across a bilayer (Fig. 12-14*a*), a process termed **transverse diffusion** or a **flip-flop,** is an extremely rare event. This is because a flip-flop requires the polar head group of the lipid to pass through the hydrocarbon core of the bilayer. The flip-flop rates of phospholipids, as measured by several techniques, are characterized by half-times that are minimally several days.

In contrast to their low flip-flop rates, *lipids are highly mobile in the plane of the bilayer* (**lateral diffusion,** Fig. 12-14*b*). The X-ray diffraction patterns of bilayers at physiological temperatures have a diffuse band, centered at a spacing of 4.6 Å, whose width is a measure of the distribution of lateral spacings between the hydrocarbon chains in the bilayer plane. This band, which resembles one in the X-ray diffraction patterns of liquid paraffins, is indicative that *the bilayer is a two-dimensional fluid in which the hydrocarbon chains undergo rapid fluxional (continuously changing) motions involving rotations about their C—C bonds.*

The lateral diffusion rate of lipid molecules can be quantitatively determined from the rate of **fluorescence recovery after photobleaching (FRAP;** Fig. 12-15). A fluorescent group **(fluorophore)** is specifically attached to a bilayer component, and an intense laser pulse focused on a very small area (~3 μm^2) is used to destroy (bleach) the fluorophore there. The rate at which the bleached area recovers its fluorescence, as monitored by fluorescence microscopy,

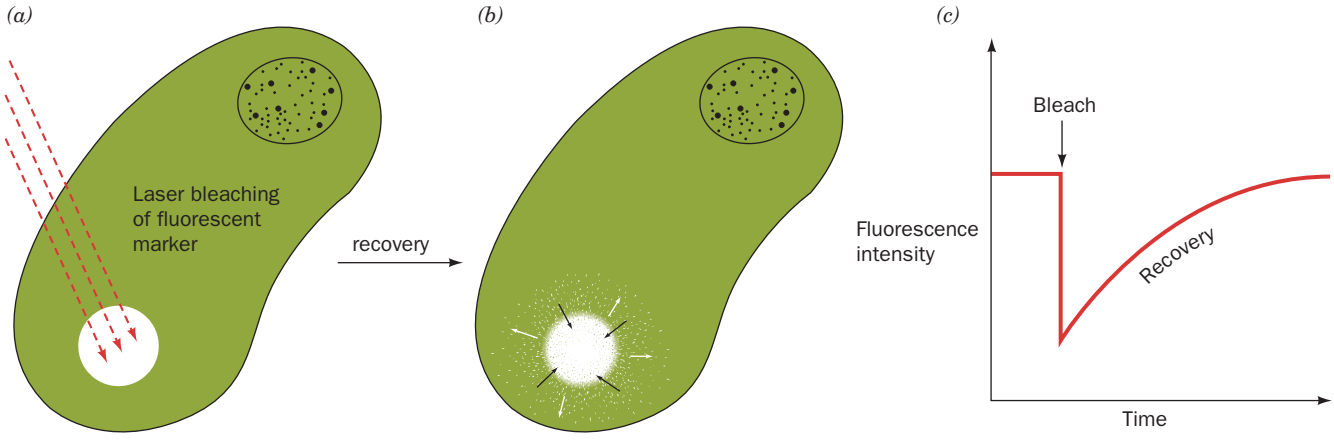


Figure 12-15 **The fluorescence recovery after photobleaching (FRAP) technique.** (*a*) An intense laser light pulse bleaches the fluorescent markers (*green*) from a small region of an immobilized cell that has a fluorescence-labeled membrane component. (*b*) The fluorescence of the bleached area, as monitored by fluorescence microscopy, recovers as the bleached molecules laterally diffuse out of it and intact fluorescence-labeled molecules diffuse into it. (*c*) The fluorescence recovery rate depends on the diffusion rate of the labeled molecule. **See Guided Exploration 11: Membrane structure and the fluid mosaic model**

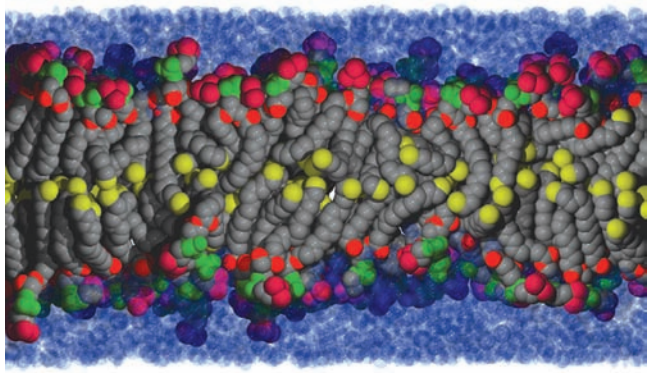


Figure 12-16 **Model (snapshot) of a lipid bilayer at an instant in time.** The conformations of dipalmitoylphosphatidylcholine molecules in a bilayer surrounded by water were modeled by computer. Atom colors are chain and glycerol C gray except terminal methyl C yellow, ester O red, phosphate P and O green, and choline C and N magenta. Water molecules are represented by translucent blue spheres (those near the bilayer appear dark because they overlap head group atoms). [Courtesy of Richard Pastor and Richard Venable, NIH, Bethesda, Maryland.] **See Guided Exploration 11: Membrane structure and the fluid mosaic model**

indicates the rate at which unbleached and bleached fluorescence-labeled molecules laterally diffuse into and out of the bleached area, respectively. Such observations indicate, as do magnetic resonance measurements, that lipids in bilayers have lateral mobilities similar to those of the molecules in a light machine oil. Lipids in bilayers can therefore diffuse the 1-μm length of a bacterial cell in ~1 s. Methods for tracking the motions of single molecules in membranes have also been developed that link the molecule of interest to a small latex bead, colloidal gold particle, or fluorescent group, and then observe the movement of the label using high speed video techniques.

Molecular dynamics simulations (Section 9-4a) of lipid bilayers (Fig. 12-16) indicate that their lipid tails are highly conformationally mobile due to rotations about their C—C bonds. However, the viscosity of these tails sharply increases closer to the lipid head groups because their lateral mobilities are more constrained by interactions with the more rigid head groups. Note that the methyl ends of the tails from opposite leaflets of the bilayer are frequently interdigitated rather than forming entirely separate layers, as Fig. 12-14 might be taken to suggest. This is particularly true in biological membranes because their various lipid molecules have tails of different lengths and/or are kinked due to the presence of double bonds. Molecular dynamics simulations also indicate that a lipid bilayer is flanked by several layers of ordered water molecules. Moreover, as Fig. 12-16 indicates, water molecules commonly penetrate well below the level of the head groups and glycerol residues. Hence, *a lipid bilayer typically consists of an ~30-Å-thick hydrocarbon core bounded on both sides by ~15-Å-thick interface regions containing rapidly fluctuating conglomerations of head groups, water, glycerol, carbonyl, and methylene groups.*

b. Bilayer Fluidity Varies with Temperature

As a lipid bilayer cools below a characteristic ***transition temperature,*** *it undergoes a sort of phase change, termed an* ***order–disorder transition,*** *in which it becomes a gel-like solid* (Fig. 12-17); that is, it loses its fluidity. Below the transition temperature, the diffuse 4.6-Å X-ray diffraction band characteristic of the lateral spacing between hydrocarbon chains in a liquid-crystalline bilayer is replaced by a sharp 4.2-Å band similar to that exhibited by crystalline paraffins. This indicates that the hydrocarbon chains in a bilayer become fully extended and packed in a hexagonal array as in crystalline paraffins.

The transition temperature of a bilayer increases with the chain length and the degree of saturation of its component fatty acid residues for the same reasons that the melting temperatures of fatty acids increase with these quantities. The transition temperatures of most biological membranes are

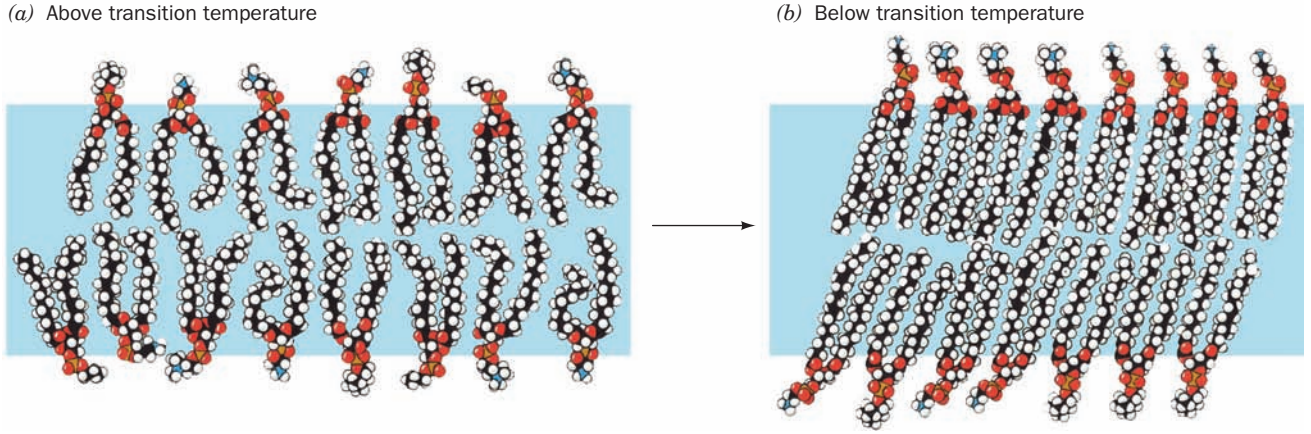


Figure 12-17 **Order–disorder transition in a lipid bilayer.** (*a*) Above the transition temperature, both the lipid molecules as a whole and their nonpolar tails are highly mobile in the plane of the bilayer. Such a state of matter, which is ordered in some directions but not in others, is called a liquid crystal. (*b*) Below the transition temperature, the lipid molecules form a much more orderly array to yield a gel-like solid. [After Robertson, R.N., *The Lively Membranes, pp.* 69–70, Cambridge University Press (1983).]

Table 12-3 **Lipid Compositions of Some Biological Membranes**[a]

Lipid	Human Erythrocyte	Human Myelin	Beef Heart Mitochondria	*E. coli*
Phosphatidic acid	1.5	0.5	0	0
Phosphatidylcholine	19	10	39	0
Phosphatidylethanolamine	18	20	27	65
Phosphatidylglycerol	0	0	0	18
Phosphatidylinositol	1	1	7	0
Phosphatidylserine	8.5	8.5	0.5	0
Cardiolipin	0	0	22.5	12
Sphingomyelin	17.5	8.5	0	0
Glycolipids	10	26	0	0
Cholesterol	25	26	3	0

[a]The values given are weight percent of total lipid.

Source: Tanford, C., *The Hydrophobic Effect, p.* 109, Wiley (1980).

in the range 10 to 40°C. *Cholesterol, which by itself does not form a bilayer, decreases membrane fluidity near the membrane surface because cholesterol's rigid steroid ring system interferes with the motions of the fatty acid tails causing them to become more ordered.* However, because cholesterol does not extend into the membrane as far as most lipids, it also acts as a spacer that facilitates the increased mobility of the fatty acid tails near their methyl ends. Cholesterol also broadens the temperature range of the order–disorder transition and in high concentrations totally abolishes it. This behavior occurs because cholesterol inhibits the crystallization (cooperative aggregation into ordered arrays) of fatty acid tails by fitting in between them. Thus cholesterol functions as a kind of membrane plasticizer.

The fluidity of biological membranes is one of their important physiological attributes since it permits their embedded proteins to interact (Section 12-3C). The transition temperatures of mammalian membranes are well below body temperatures and hence these membranes all have a fluidlike character. Bacteria and poikilothermic (cold-blooded) animals such as fish modify (through lipid biosynthesis and degradation) the fatty acid compositions of their membrane lipids with the ambient temperature so as to maintain membrane fluidity. For example, the membrane viscosity of *E. coli* at its growth temperature remains constant as the growth temperature is varied from 15 to 43°C.

C. Gaseous Anesthetics Alter Neuronal Membrane Structures

Gaseous anesthetics, such as diethyl ether, cyclopropane, **isoflurane** ($CF_3—CHCl—O—CHF_2$), and the noble gas Xe, act by interfering with the transmission of nerve impulses in the central nervous system. Since the body excretes these general anesthetics unchanged, it appears that they do not act by chemical means. Rather, experimental evidence, such as the linear correlation of their anesthetic effectiveness with their lipid solubilities, suggests that these nonpolar substances alter the structures of membranes by

Table 12-4 **Compositions of Some Biological Membranes**

Membrane	Protein (%)	Lipid (%)	Carbohydrate (%)	Protein to Lipid Ratio
Plasma membranes:				
Mouse liver cells	46	54	2–4	0.85
Human erythrocyte	49	43	8	1.1
Amoeba	52	42	4	1.3
Rat liver nuclear membrane	59	35	2.0	1.6
Mitochondrial outer membrane	52	48	(2–4)[a]	1.1
Mitochondrial inner membrane	76	24	(1–2)[a]	3.2
Myelin	18	79	3	0.23
Gram-positive bacteria	75	25	(10)[a]	3.0
Halobacterium purple membrane	75	25		3.0

[a]Deduced from the analyses.

Source: Guidotti, G., *Annu. Rev. Biochem.* **41,** 732 (1972).